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REMARKS

Claims 51-93 are pending in the instant application. Reconsideration of this application in view of the amendments presented above and the remarks presented below is respectfully requested.

Information Disclosure Statement

The Examiner indicates that reference C10 of the Information Disclosure Statement filed July 14, 2003, has not been reviewed. Applicants have included a copy of reference C10 herewith, and respectfully request review of the reference.

35 USC § 112, second paragraph

Claims 71 and 72 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. In particular, the Examiner asserts that Claim 71 is indefinite due to its dependency from Claim 70. In addition, the Examiner asserts that Claim 72 lacks antecedent basis in its recital of “ $n \geq 6$.” In light of the amendments, presented above, Applicants submit that the claims are sufficiently definite and thus withdrawal of the pending rejections under 35 USC § 112, second paragraph, are respectfully requested.

Double Patenting

Claim 87 stands objected to under 37 CFR 1.75 as being a substantial duplicate of claim 88. In light of the above amendment, canceling Claim 88, Applicants respectfully request withdrawal of this objection.

35 USC § 102(e)

Claims 51-58, 60-62, 64-73, 79, 80, 82, 83, 85-89 and 93 stand rejected under 35 USC § 102(e) as anticipated by Wohlstadter et al., US Patent No. 6,066,448 (“Wohlstadter”). Wohlstadter is a continuation-in-part of USSN 08/402,076, filed March 10, 1995, which is a continuation-in-part of USSN 08/402,277, also filed March 10, 1995. Accordingly, the earliest possible priority date available for the disclosure in Wohlstadter is March 10, 1995.

In response to the rejection, Applicants are submitting herein an unsigned Declaration under 37 C.F.R. §1.131 by the inventors, Thomas J. Meade and Jon F. Kayyem, referencing experiments reflected in laboratory notebooks dated prior to March 10, 1995. A signed copy of the declaration will be forwarded as soon as possible. The declaration outlines that the invention was completed in this country prior to March 10, 1995. Accordingly, Wohlstadter is not a prior art reference, and Applicants respectfully request withdrawal of the rejections under 35 USC § 102(e).

35 USC § 103(a)

Claims 59, 63, 81, 84 and 85 stand rejected under 35 USC § 103(a) as being unpatentable over Wohlstadter in view of Kayyem et al., U.S. Patent No. 6,096,273 (Kayyem).

As discussed above, Wohlstader is not a prior art reference, and thus cannot be combined with Kayyem as described by the Examiner when making the instant rejections. Accordingly, Applicants respectfully request withdrawal the rejections under 35 USC 103(a).

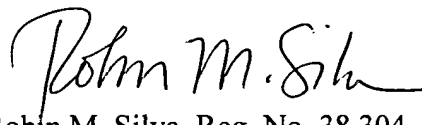
CONCLUSION

Applicants respectfully submit that the claims are in condition for allowance and early notification to that effect is respectfully requested. Please direct any calls in connection with this application to the undersigned attorney at (415) 781-1989.

Respectfully submitted,
DORSEY & WHITNEY LLP



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Suite 3400
San Francisco, California
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Robin M. Silva, Reg. No. 38,304
Filed under 37 CFR §1.34(a)



PATENT

Attorney Docket No. A-64411-2 (468267-00067)/RMS/RMK/SPL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

MEADE, *et al.*

Serial No.: 09/921,645

Filed: August 3, 2001

For: *Metallic Solid Supports Modified
with Nucleic Acids*

Group No. 1637

Examiner: Strzelecka, Teresa, E.

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, Box 1450, Alexandria, VA 22313-1450 on:

Date: 4/7/04

Signature [Signature]

Steve Lendaris

DECLARATION PURSUANT TO 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

Sir:

We, Thomas J. Meade and Jon F. Kayyem hereby declare as follows:

1. We are the inventors on the above-identified patent application and are familiar with its contents. We have also reviewed the pending claims in this application.
2. We are familiar with the Office Action mailed on November 17, 2003 wherein claims 51-69, 71, 72, and 74-93 were rejected over Wohlstadter et al. (6,066,448) which has an earliest possible priority date of March 10, 1995.
3. All of the ideas detailed in the above-identified application were contemplated in this country prior to March 10, 1995. This is evidenced by the appended documents.
4. One of the goals of the project that led to the filing of the parent application was to create a surface comprising a self-assembled monolayer with single stranded nucleic acids attached (referred herein as probes), and then to answer three questions: first, whether a solution-based complementary strand would bind to the probe; second, would a complementary strand attached to an atomic force

microscopy (AFM) tip bind to the probe, and if so; third, whether or not we could determine the force necessary to "tear apart" the duplex.

5. The experiments started out with the synthesis of the monolayer portion using an $\text{HO}-(\text{CH}_2)_{16}-\text{OH}$ to form a molecule with a protected sulfur group (for attachment to a gold surface) on one end, to which a phosphoramidated nucleic acid was attached. The experiments proceeded with the coating of a gold surface with this monolayer-forming material. A photolithographic mask, with 8×8 micron squares on it, was then used to cover the gold surface. The surface was then exposed to a photoactivated agent and a mercury arc lamp which resulted in the ablation nucleic acids from the squares not covered by the mask. We then added a fluorescent complement to the surface, and viewed it under a confocal microscope. This resulted in a pattern of "light", e.g. fluorescent, background, where the fluorescent solution based probes were found, and "dark" squares, where the surface-bound single stranded nucleic acid had been ablated off, and therefore no fluorescent probe was detected. A montage of several of these images, made over the course of the experiments, is attached as Exhibit A.
6. With regard to timing of these experiments, the documents attached as Exhibit B are pages from my notebook detailing the synthesis of some of the compounds used in these experiments. (Please note that all experiments not relevant to the present discussion have been redacted, as have all dates.) For example, page 136 documents the conversion of the $\text{HO}-(\text{CH}_2)_{16}-\text{OH}$ molecule to the asymmetrical $\text{HO}-(\text{CH}_2)_{16}-\text{OAc}$ needed for further reactions. The bottom of page 139 and the top of page 140 show the synthesis of the protected thiol- $(\text{CH}_2)_{16}-\text{OH}$ molecule. the top of page 141 shows the reaction of the protected thiol- $(\text{CH}_2)_{16}-\text{OH}$ molecule added to a phosphoramidite moiety. In conclusion, the invention was completed in this country prior to March 10, 1995.
7. We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the making of willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

willful statements may jeopardize the validity of the application or any patent
issuing thereon.

Date: _____
Thomas J. Meade

Date: _____
Jon F. Kayyem

UNSIGNED



EXHIBIT A

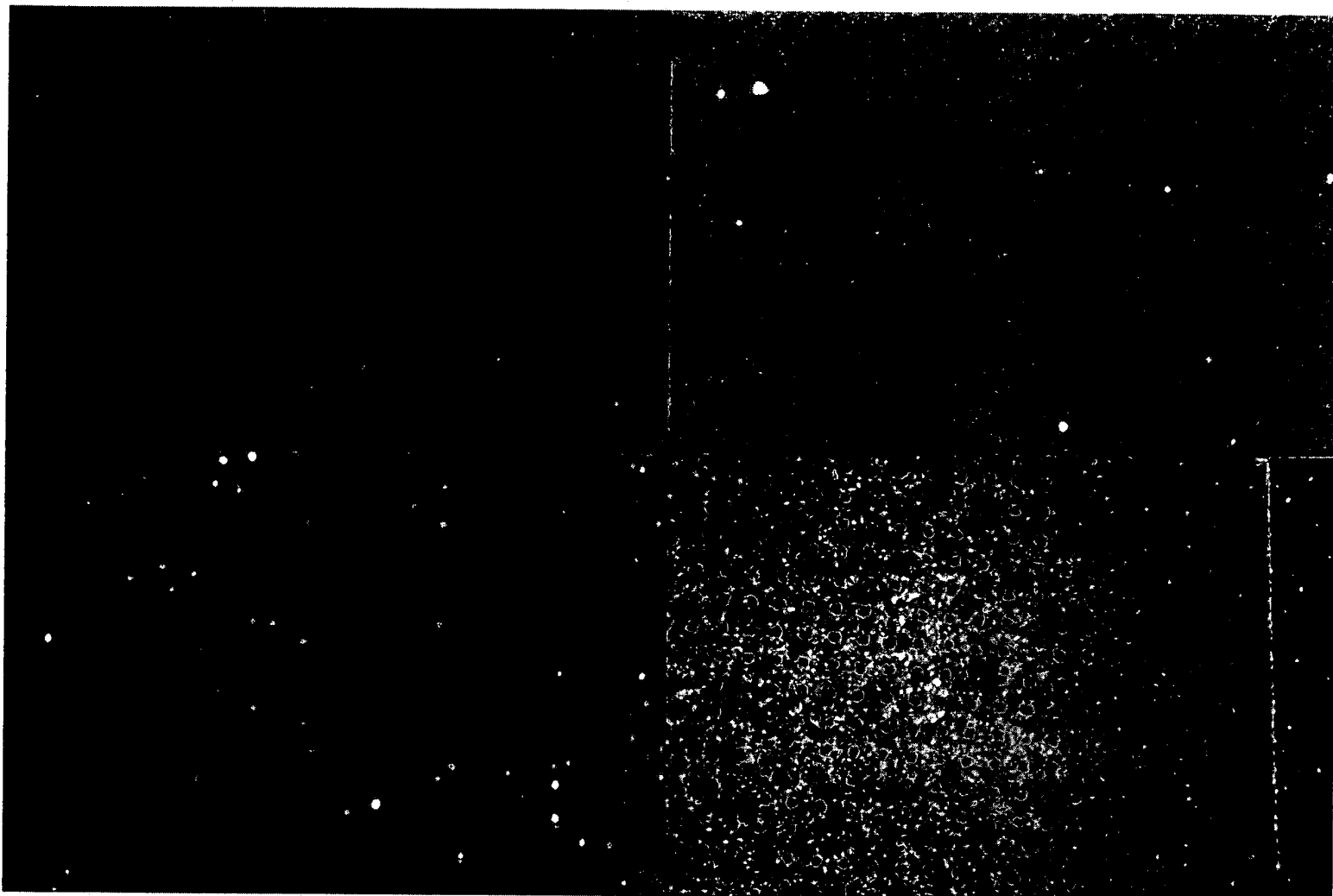
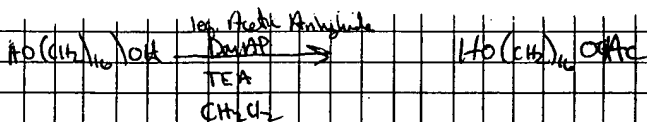




EXHIBIT B



0.5 gr of $HO(CH_2)_{10}OH$ (mw 258.45; 1.9×10^{-3} moles) was placed in a small round bottom and 20mls of CH_2Cl_2 added along with 0.05 equiv (9.7×10^{-5} moles (12.7 or 11.8 mg) and 1.4 equiv of TEA (4.7 ml) and 1 equiv of Acetic Anhydride (mw 102.9; d=1.08) [10.57 mls/liter] or 173 ml.



20.50 ether/hexane

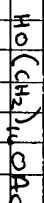
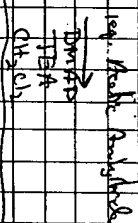
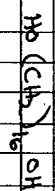
↓ continuous extract
Diol DMAP
Std Rec.

50/50
ether/hexane

Rec. 2

2.05 gr (7.93×10^{-3} moles) was placed in a 100 ml RBF and 60 mls of CH_2Cl_2 added along with 0.05 equiv DMAP (48 mg) and 1.4 equiv of TEA (1.7 mls) and 6.95 ml of Acetic Anhydride

Reaction 3

3.0 g of $\text{HO}(\text{CH}_2)_{10}\text{OH}$

70 mg DMAP

2.4 g TEA

102 mg of Acetic Anhydride

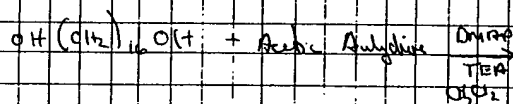
100 ml RBF / 60 ml of CH_2Cl_2

Rec. 3
 1.4 g of $\text{HO}(\text{CH}_2)_{10}\text{OH}$
 73 mg DMAP
 1.2 ml TEA

484 ml Acetic Anhydride

Rec. 3
 9.875 g
 2.4 g TEA
 102 mg
 100 ml RBF
 60 ml CH_2Cl_2

138

Rec 3

1.37 gr of Diol was dissolved in 25 ml of dry CH_2Cl_2 . 0.5 equiv
 of DMAP (32.33 mg) was added and 1.4 equiv of TEA (1.14 ml)
 and 475 μl of Acetic Anhydride.

Tube 72

→ mono
acetate

50 ml solvent from 7 min dist. 0.9
 12 mm monoacetate = 1.5
 35 mm diacetate = 0.7

The flash column was

20 250 ml of silica:

1.2 liters of 180/20 hex/Ether
 was passed through and the
 gradient pushed up to 50

55, 45, After tube 72

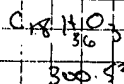
it broke and 200 ml of 50/50

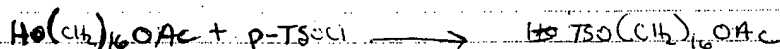
to remove all the product

→ ?

→ mono acetate

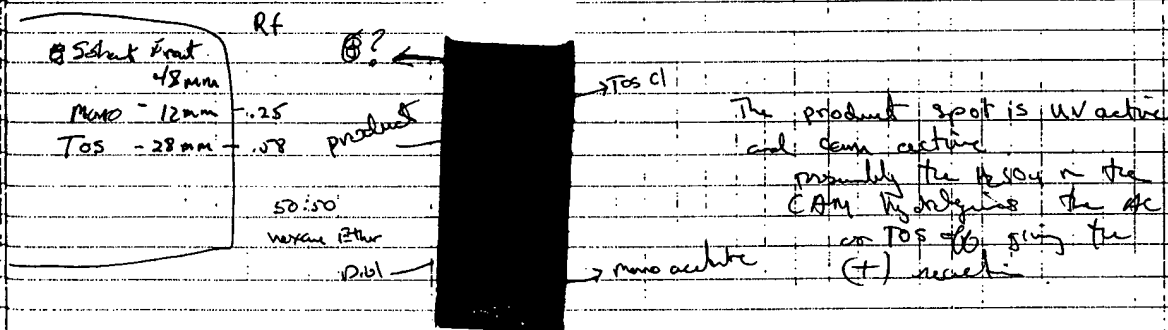
→ Diol 50/50 Ether/hexane





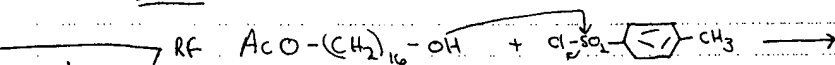
Expt + Expt page 1180

500mg (1.7×10^{-3} moles) of $\text{HO}(\text{CH}_2)_{16}\text{OAc}$ in dry pyridine and in 50 ml ethylmethyl ether and cooled to 0°C . p-TsCl (190.65) in 1 molar excess (or 634 mg) with a string bar and allowed to proceed for 40 hrs. The solution was poured into a beaker with 200 ml of ice water and Section A fluid at the bottom. The solution was stirred in heptane (20) and returned to

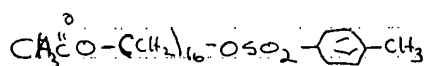


dryness. $^1\text{H-NMR}$ is consistent with the proposed structure

Repeat



Solvent front = 47
TOS 215
Rf: 0.59

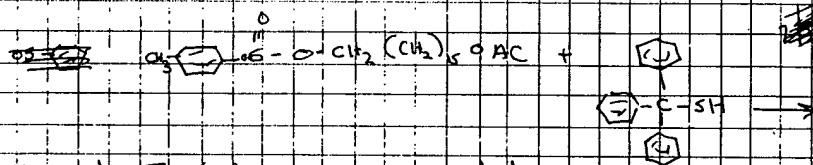


TOS 17-36 mm

$$\text{C}_{25}\text{H}_{42}\text{O}_5 = 454.72$$

$$\text{C}_{25}\text{H}_{42}\text{O}_5 = 438.72 + 15.958$$





370 mg of $\text{TSC}(\text{CH}_2)_{16}\text{OAc}$ was dissolved in 10 ml of DMF, and thoroughly degassed on the vac. line ($n_D = 1.45472$ or 8.1×10^{-4} moles)

NaOH - 40

- Triphenyl methyl mercaptane { n_D 276.610; 0.95 equiv = 7.7×10^{-4} moles = 213 mg.5 }
- 31.8 mg of NaOH (.93 equiv) in $\frac{\text{more of } \text{H}_2\text{O}}{180 \text{ ml}}$

5 ml of ethanol was degassed on the vac line and 213 mg of triphenyl methyl added. @ 180 ml of degassed NaOH in H_2O was added via syringe under Ar . To this solution 370 mg of $\text{TSC}(\text{CH}_2)_{16}\text{OAc}$ in DMF/EtOH was added and the solution degassed.

After treatment with NaOH in MeOH →

Excess triphenyl methyl

$\text{Triphenyl-S-(CH}_2)_{16}\text{OAc?}$

$\text{Triphenyl-S-(CH}_2)_{16}\text{OH}$

DMF

ST-S.F.

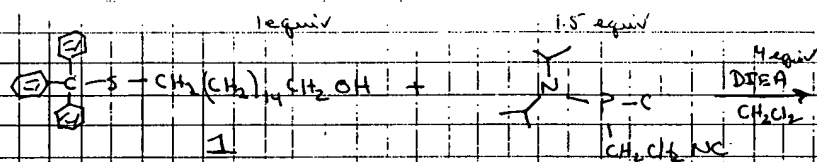
Product

Solvent = 48 mm
Triphenyl-OH = 19 mm
or Rf: 4

.84

The material was taken up in MeOH/MeOH 6/4

90.20



$\text{C}_{34}\text{H}_{48}\text{OS}$ - MW 504.87

• 220 mg of 1 = 4.4×10^{-4} moles

• 6.5×10^{-4} moles of phosphoramidite 236.68 $d = 1.061$ $M = 4.48$ mL

574 mg of 2 = 1.8×10^{-3} moles DIEA MW 129.25 $\frac{0.742}{129.25} = 5.74 \text{ mg}$

220 mg of 1 was slurried in 15 mL of dry CH_2Cl_2 and immediately 326 μL of DIEA was added. 145 μL of O-acyl phosphoramidite was added dropwise. After 30 min and additional 50 μL of O-acyl reagent was added.

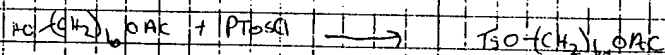
Note:

TEA MUST be present during flush!

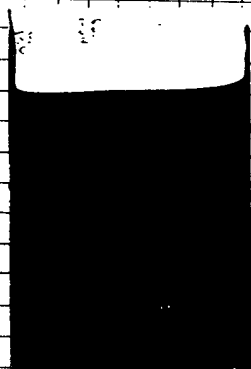
\therefore At the addition of 1% TEA to the mobile phase, drop the diethyl ether

Trifluoromethane

142



51 gr of monoac product was stirred in 30 ml of dry pyridine and cooled to 0°C. 650 mg of TOSCl was added and the reaction mixture allowed to proceed for 40 hrs.



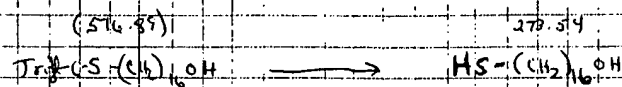
→ monoac

Note:

Must use FRESH TBOC

Total yield 150 mg

50/50 Ethyl/Hexane



200mg 11mg of Trityl-S-(CH₂)₁₆OH (2.13 x 10⁻⁵ moles)

276.40
- 101
275.39
+
516.89

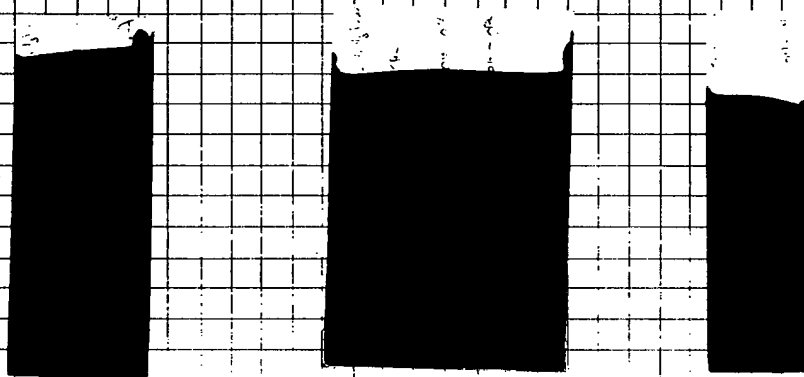
0.3 mmol
100 mM TEAE
100 mM AgNO₃
140 mM DTT

1mg dissolved in MeOH (5mls and adding TEAE buffer)

1ml of AgNO₃ solution - 30 min

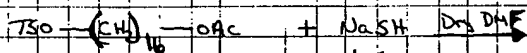
1ml of DTT - 30 min (154.2 mm) 1525 mm 1ml = 1M

1.1 ml of 100 mM DTT or 1.1 ml of 1M DTT
1.1 ml of 100 mM AgNO₃ or 1.1 ml of 1M AgNO₃



NMR says NR. The majority of isolated material is starchy material.

144



1.5 equiv
2 equiv

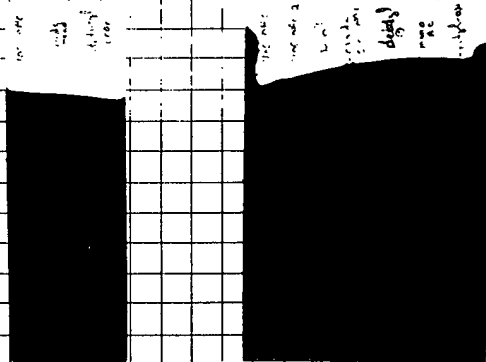
O

MW = 454.72

or 3.3×10^{-4}
or 18.4 μg

NaI + Anhydrous Acetone

EtOH + AgNO₃
reaction



NMR reveals that the spot identified ① is from the reaction of triethyl-OH with AgNO₃ did not occur

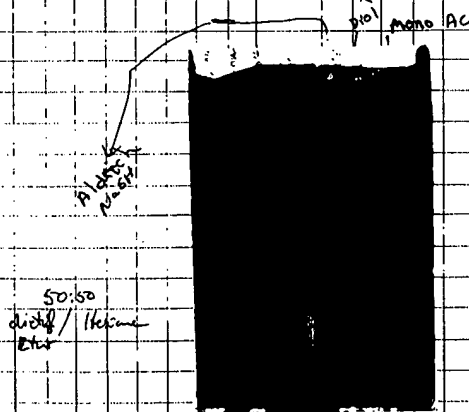
NMR = triethyl-OH NMR

50:50
Dichloroethane:Hexane

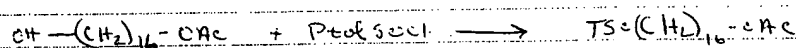
NMR

$\approx 75 \text{ mgs}$ starting material = 1.7×10^{-4} moles $\times 1.2$ equiv of NaOH

2×10^{-4} moles NaOH or 8 mgs dissolved in MeOH

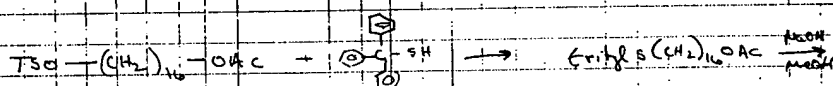


with "Anhydrous" NaOH



2 x 250mg or 8.5×10^{-4} moles in 15 ml of dry pyridine is cooled to 0°C in an ice bath. 320 mg of TSOCl is added and the reaction allowed to proceed @ 4°C . The product (brownish tint) is poured into a beaker with amounts of ice water, stirred for 10 min and filtered. The solid is washed with water and dissolved in pet ether and charcoal added with stirring. The mixture was filtered and dried (400mg total).

2.4 hours is not long enough, some ortho remains



325 mg (mw = 454.72) or 7.15×10^{-4} moles was dissolved in 10 ml of dry DMF and degassed. Triphenyl methylmercaptan (mw 276.4) with

1.1 equiv (7.86×10^{-4}) or 217 mg and 1.05 equiv of NaOH or 30 mg was dissolved in 150 ul of H_2O and degassed.

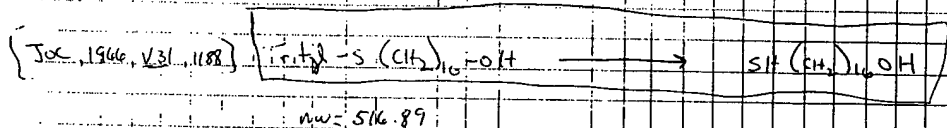
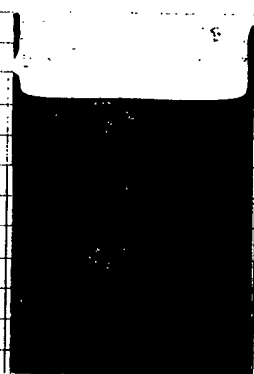
The triphl SH was dissolved in 5 ml of EtOH and degassed. The NaOH was added via syringe to the TSC-OAc, ~~added~~. The reaction was repeatedly degassed and allowed to proceed for 12 hrs.

The reaction was TLC, and NaOH/MeOH added to develop.

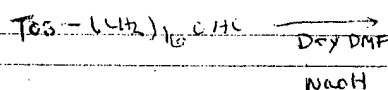
⇒ Flash → phosphoramidite

26

EtOAc

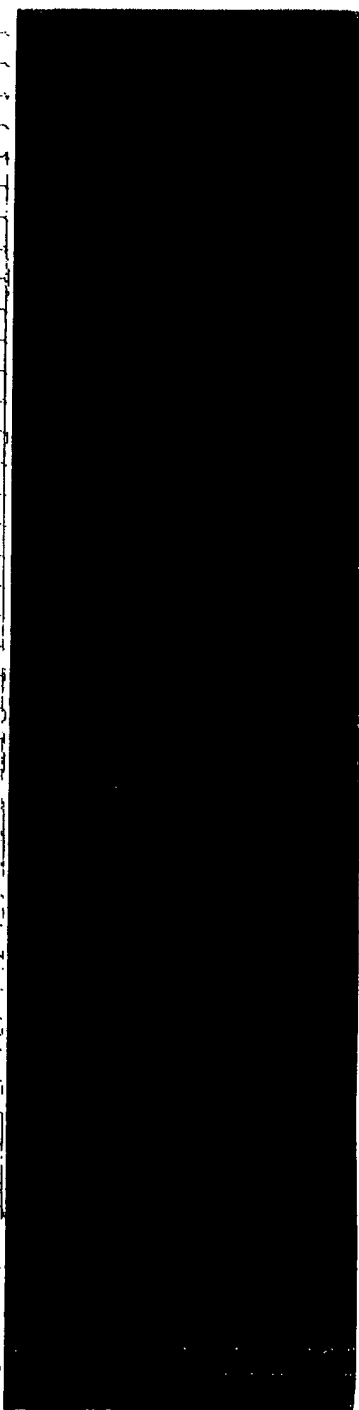
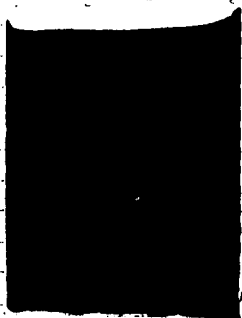
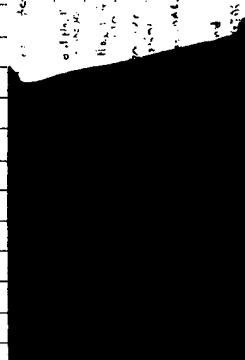
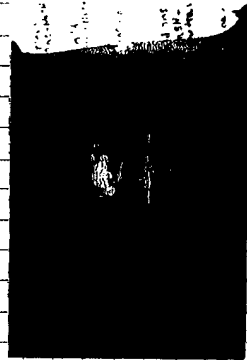
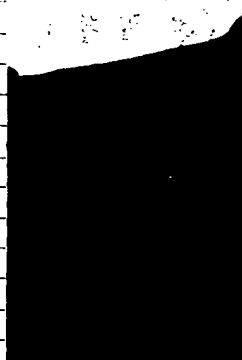


25 mg (4.8×10^{-5} moles) was dissolved in ^{glacial} acetic acid (0.15 ml) and .38 mls of 1N HCl added. The reaction was allowed to stir for 1.5 hrs and 75°C. Upon addition of 100, the emulsion formed a white ppt. immediately. After warming for ~ 5 min the ppt. redissolved with the resulting solution slightly cloudy.

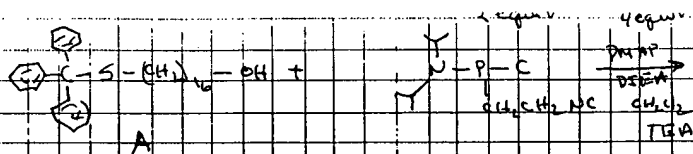


MW = 454.72

- ① 65 mg of the Tos derivative was dissolved in 5 ml of dry DMF.
 1.4×10^{-4} moles \times 2 equiv of NaSH or 2.9×10^{-4} moles or $\frac{17}{16}$ mg
 of NaOH is dissolved in 1 ml of dry MeOH. The NaSH
 (in 2 ml of dry MeOH) was degassed and the MeOH added.
 This solution is added (via syringe to the dry DMF Tos
 products.



148



170 mg of A = 3.4×10^{-4} moles

151 μl or 2 equiv. of phosphoramidite

240 μl of DIEA

170 mg of A was dissolved in 45 ml of CH_2Cl_2 and

240 μl of DIEA added. 151 μl of phosphoramidite were added

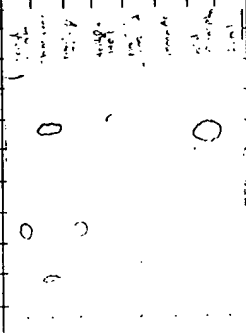
dropwise over 30 min.

The column was run with

90:10:0.5

Hex: Diethyl ether: TEA

200 ml of solvent



Product \rightarrow

○ ○

major \rightarrow

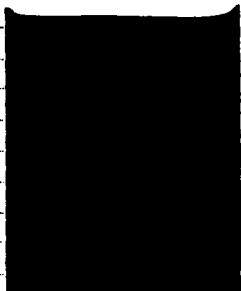
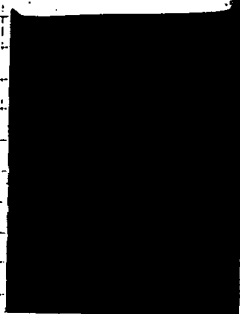
○

50:50:2

50:50:1%
TEA

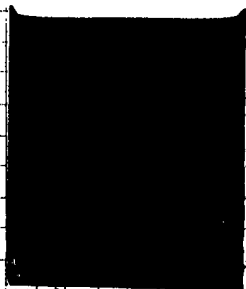
isolated
 ≈ 125 mg
methylol

Sample = 40 min

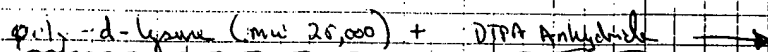


- A plate using ~~Ammonium~~ Ammonium Calcium Nitrate revealed on the presence of DTT.

After cooling a white ppt. formed and the solution was dissolved in 100 mL of CH_2Cl_2 . Na_2CO_3 (5M, pH 8) was used to wash the CH_2Cl_2 . The reaction was conducted until the water layer was pH 8. The solution was then washed with pH 7 buffer, returned to dryness.



→ Before base wash
↳ After washing (neutralized) with pH 8 Na_2CO_3



Poly lysine was dissolved in D_2O and (20 mgs in 2 mls) and applied to a PD-10. An additional $1/2$ ml was added and 3 mls total collected from each of 3 PD-10 columns. The recovered dried material (≈ 65 mgs) was divided into 3 reaction vessels of 22 mgs each.

The following ratios will be prepared

50X
100X
200X

(See page 100 + 101)

0.1 mM Poly-d-lysine:

$$22 \text{ mgs } (8.6 \times 10^{-7} \text{ moles}) \times \boxed{8.6 \text{ mls}} = 0.1 \text{ mM}$$

$$50 \times \text{DTPA Anhydride} \quad \text{or } \{4.3 \times 10^{-5} \times 357.3 = 15.4 \text{ mgs}\}$$

$$100 \times \quad \text{or } \{8.6 \times 10^{-5} \times 357.3 = 30.7 \text{ mgs}\}$$

$$200 \times \quad \text{or } \{1.72 \times 10^{-4} \times 357.3 = 61.5 \text{ mgs}\}$$

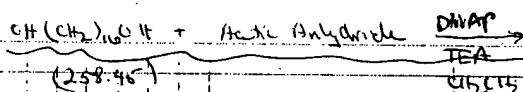
0.5 M H_2CO_3 buffer, pH 9.5

Only the 200 reaction showed any change in pH (e.g. $\rightarrow 9.0$)

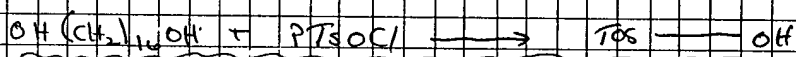
The reaction were ~~not~~ speed record to ~~change~~

$$400 \times \quad 35.2 \text{ mgs or } 1.4 \times 10^{-6} \text{ moles}$$

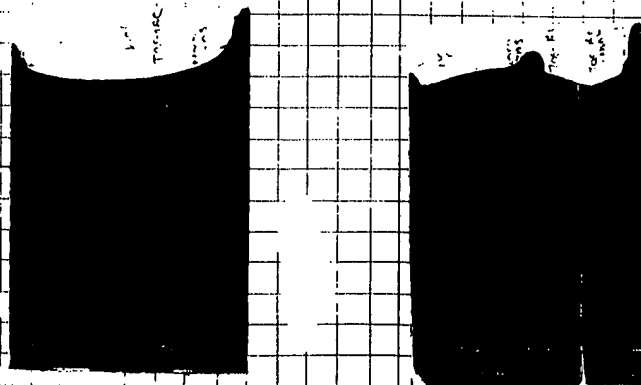
200 mgs of DTPA Anhydride in 14 mls pH 9.5 H_2CO_3 Buffer



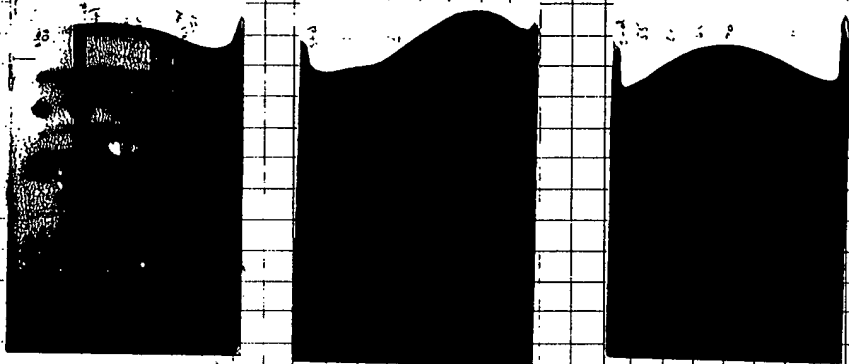
3 gr of diol was stirred in 50mls of Dry CH_2Cl_2 and
~~20~~ 71 mg of DMAP added along with 2.5 mls of TEA
 and 1.04 mls of Acetic Anhydride



2 gr of $\text{OH(CH}_2\text{)}_{10}\text{OH}$; 7.74×10^{-3} moles) was ^{stirred} dissolved in 250 mls
 of dry pyridine and cooled to 5°C . TsCl (150.65) equiv
 = 1.48 gram was added and the reaction allowed to proceed
 for

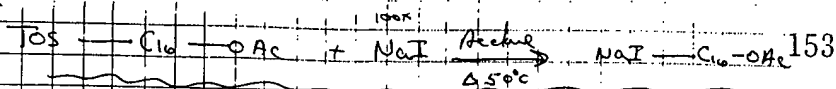


TOS—C16—OAc, Purification



The water/py solution is evaporated to dryness and stirred in heptane/diethyl ether and evaporated 3 times. The solid is dissolved in 50% 1,2-ethanediol:hexane and filtered. The solution is applied to a column of 5% hexane:EtOH. Plate 2 reveals impurity. Future: Run 90:10 to start and move up to 80:20

See page 139 for prep.



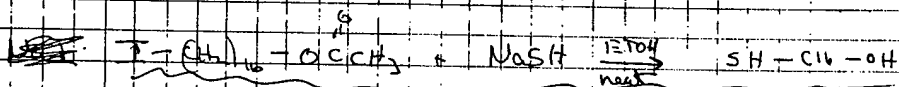
180 mp

450

0.37 g of Ios - OAc (MW = 454.72) or 5.2×10^{-4} moles is dissolved in

anhydrous Acetone and a 100x or 5.2×10^{-2} moles of ~~NaI~~ (MW 149.89)

of NaI added. The reaction is followed by TLC. After 15 min a crystalline material began to pop out.



50 mg of $\text{I} - (\text{CH}_2)_6 - \text{O} - \text{C}(\text{CH}_3)_3$ was dissolved in ~5 mL of EtOH.

75 mg of NaSH (10x) was

slurried in 20 mL of EtOH

and the iodide added slowly.

and kept at ~60°C for 2 hrs.